Acta Biologica Szeged. *22* (1—4), pp. 73—78 (1976)

EFFECTS OF SATURN-50 AND SYNPRÁN-111 HERBICIDES ON THE MEMBRANE-BOUND ATP-ASE ACTIVITY OF ROOTS AND YOUNG SHOOTS OF RICE

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(Received May 20, 1975)

Abstract

The effects of two herbicides for rice: the Saturn—50 (benthiocarb) and the Synprán—111 (2, 4, 5—T+DCPA) on the Na^+ and K^+ activated ATP-ase of roots and young shoots of rice were investigated. The summary of our experimental results is as follows.

1. The activity of the enzyme prepared from plants which were grown in a water-culture containing $5 \cdot 10^{-4}$—10^{-7} M herbicide was compared to control values: it can be stated that the root-ATP-ase became 50 per cent more active under the influence of 10^{-6} M Saturn—50, while the 10^{-7} M Synprán—111 resulted in a stimulation of 40 per cent. The basic activity was inhibited by the $5 \cdot 10^{-4}$ M Saturn—50 by more than 70 per cent, and by the Synprán—111 of a similar concentration it was decreased approximately by 80 per cent.
2. In the case of root ATP-ase of rice preincubated with $5 \cdot 10^{-4}$—10^{-10} M Saturn—50, the 10^{-8} M Saturn—50 casued an increase of about 50 per cent in the activity, the $5 \cdot 10^{-4}$ M concentration, however, inhibited the enzyme activity by 85 per cent. The root ATP-ase was not stimulated by the Synprán—111 even at a low concentration but the $5 \cdot 10^{-4}$ M resulted in about 75 per cent inhibition.
3. On the investigation of the herbicide sensitivity of the young shoots ATP-ase we found that the basic activity was increased by nearly 30 per cent by the 10^{-9} M Saturn—50, while an inhibition of more than 65 per cent resulted from the $5 \cdot 10^{-4}$ M. The enzyme obtained from the shoot was more sensitive to the Synprán—111, i. e. the 10^{-7} M resulted in a stimulation of nearly 50 per cent and the $5 \cdot 10^{-4}$ M inhibited by 80 per cent.

Introduction

The wide-spread application of herbicides, fungicides, and insecticides raises the question of how these biologically active substances act on cultivated plants. ZSOLDOS and MÉCS (1974) showed by their experiments that the uptake of ions in the root is inhibited by some rice herbicides: Saturn, Synprán, Dacthal, and the fungicide Kitazin in higher concentration; while, in case of a low concentration, a significant increasing activity can be observed. Investigations of the free amino acid and the ion efflux prove that the effects of the above substances can frequently be traced back to the permeability of the membrane. Therefore, we found worth studying the question of how certain herbicides acted on one enzyme of the cell membrane, namely, on the ATP-ase depending upon Na^+ and K^+ which acts in the presence of Mg^{++}.

Recently, it is proved by more and more experimental data that the plant ATP-ases can be brought into connection with the uptake of ions and the transport processes of the plants, similarly in the case of the functions of animal ATP-ases (LEIGHT, 1975; LEONARD, 1973; HODEGS, 1972). KASAMO and his co-workers (1973, 1974) have already investigated the effects of some biologically active substances on the ATP-ases of different cell fractions. They observed that, in low concentrations, the active ingredients stimulated the activity of the "transport enzyme", and so the plant transport processes.

In the course of the chemical processing in agriculture, more and more effective agents have been used. Therefore, it is important both theoretically and experimentally to study the interaction among the herbicides — biologically active substances and the ATP-ases bound to the cell membranes, respectively.

Materials and Methods

1. Growing experimental plants:
Rice plants *(Oryza sativa* L. var. *japonica)* grown in water culture were used for every experiment. The seeds were allowed to swell in tap water for 5 hours, then progerminated on a wet filter paper in a thermostat at 22 °C. Then the seeds were sown in a growing glass covered with a steel net and grown in a light of 10 000 Lux intensity for 16 hours per day. The culture fluid was $5 \cdot 10^{-4}$ M $CaSO_4$ at a temperature of 20—22 °C, pH value 6,5. The investigations were carried out with 7—8 day-old plants.
2. Herbicides used:
The effect of a Japanese product, Saturn—50 and the Hungarian Synprán—111 on rice plants was studied. The Saturn—50 which is a herbicide suitable above all for preemergent but also postemergent treatment contains 50 per cent active ingredient S-(-4-chlorobenzyl)-N, N-diethiolcarbamate. The Synprán—111 is a leaf herbicide of contact and systemic activity; it consists of 3,4-dichloropropion anilide and 2,4,5-trichlorophenoxyacetic acid amyl ester with 38,6 per cent active agent.
3. Preparation of the ATP-ase:
The different vegetal parts cut into pieces were homogenized with 4 ml of extracting mixture to every gram of fresh weight (0,25 M saccharose, 0,025 M Tris, 0,003 M EDTA). The crude extract was pressed through gauze and centrifuged under cooling: with 1500 g for 10 minutes, the supernatant layer with 4000 g for 40 minutes, the supernatant layer with 15.000 g for 30 minutes, and, finally, with 100.000 g for 90 minutes. The precipitation obtained at the last step was suspended with a medium of twice the volume (0,25 M saccharose, 0,025 M Tris-HCl buffer, pH 7,0). The enzyme extract obtained could be stored for 6 days without any decrease in its activity.
4. Measurement of the activity of ATP-ase:
The activity of enzymes was given in the μM value of inorganic phosphate released by 1 mg of protein during 60 minutes. The quantity of phosphate was measured by FISKE's and SUBBA-ROW's method; the calibration curve was made with KH_2PO_4.
The quantity of protein was measured according to LOWRY and his co-workers, the calibration curve was made with bovine albumin. The incubation mixture of the enzyme was composed in accordance with every single epxeriment. The optimum conditions for the enzyme activity were meaºured out in the course of the pre-examinations. The mixture contained the following substances (for 1 ml of its volume): 0,025 M Tris-HCl buffer, pH 7,2; $5 \cdot 10^{-3}$ M NaCl; $5 \cdot 10^{-3}$ M KCl; $5 \cdot 10^{-4}$ M $MgCl_2$; 100—200 μg protein. The reaction was started by adding $5 \cdot 10^{-3}$ M Tris-ATP, then incubated at 30 °C for 10 minutes, and, finally, it was stopped with 0,5 ml of TCA of 40 per cent. In the course of experiments with herbicides there was a pre-incubation with an active ingredient of suitable concentration for 30 minutes. The blank test was measured with boiled enzyme.
Every experiment was carried out together with three parallel ones, and repeated three times at least.

Results and Discussion

By means of experiments performed in water culture it could be stated that the two herbicides we investigated, the Saturn—50 made in Japan and the home production, the Synprán—111 used in a wide range, had, strongly diluted, a stirring effect on the rice plant and on the (Na^+, K^+) ATP-ase prepared from the root. This result, is valuable because the two herbicides are different in their mechanisms of action selectivities and chemical structures. It can be supposed, on the basis of these results, that, in a favourable concentration, these herbicides stimulate the development of plants by increasing the efficiency of metabolism in a complex manner. On the other hand, it is presumable that the above herbicides alter the ATP-ase configuration of the root membrane in such a way that an enyzme of higher activity can be obtained.

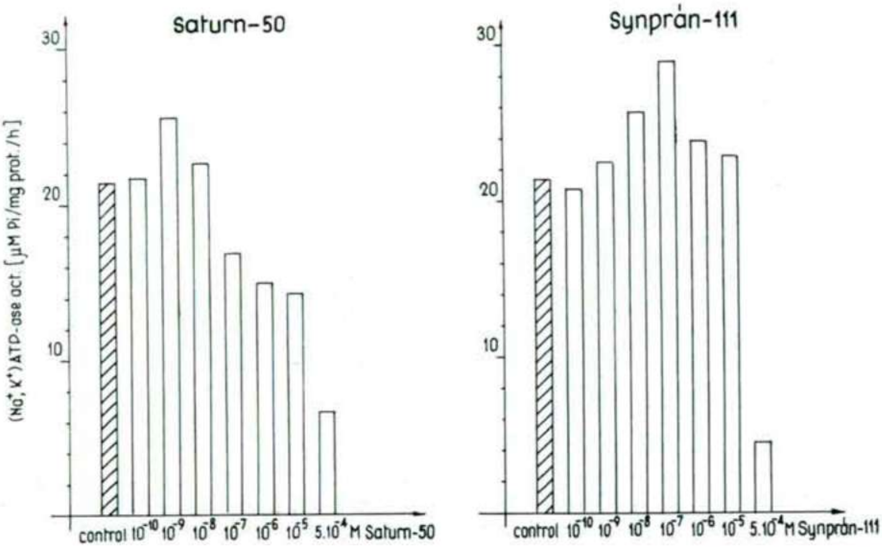

Fig. 1. The change of activity of the ATP-ase prepared from the rice roots which were grown in a culture containing herbicide.

Fig. 1 shows the change of activity of the (Na^+, K^+) ATP-ase prepared from the roots of plants which were grown in a water culture containing herbicide. The concentration of $5 \cdot 10^{-4}$, 10^{-5} M inhibited the activity of a prepared enzyme. This fact is in accordance with the observations that the uptake of ions, the respiration, and in general the vital processes become slower in the case of a higher concentration. The Saturn—50 of 10^{-6} M resulted in a significant increase in the activity, and in the case of Synprán—111 the 10^{-7} proved a stimulating concentration.

The next two Figures show the herbicide-sensitivity of ATP-ase prepared from the roots (Fig. 2) and young shoots (Fig. 3) of plants grown in cultures which did not contain any herbicide. According to Fig. 2, the basic activity increased from 19,9 to 28,7 in the presence of Saturn—50 of 10^{-8} M which means a stimulation of about 50 per cent. By comparing the definite increasing activity of the Saturn to the change caused by the Synprán—111, it can be established that this herbicide does not result in significant increase. In higher concentrations both herbicides inhibited the activity of ATP-ase.

Fig. 3 contains comparative results concerning the herbicide-sensitivity of the ATP-ase in the microsome fraction of young rice shoots. It has been found that the

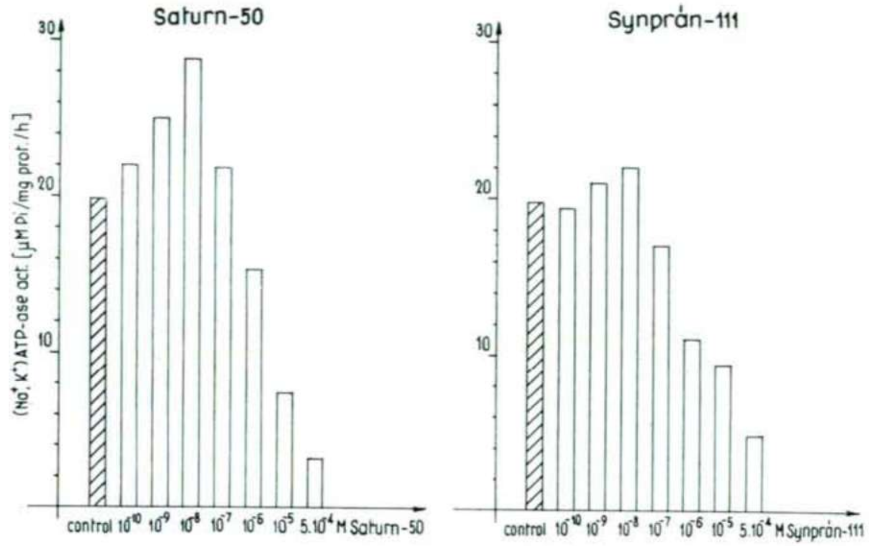

Fig. 2. Herbicide-sensitivity of ATP-ase prepared from the rice roots.

leaf herbicide Synprán—111 influences the shoot enzyme to a much greater extent than the Saturn—50. The concentration of 10^{-8} M but especially the Synprán—111 of 10^{-7} M resulted in significant stimulation (the basic activity of 21,4 increased to 28,6), and the concentration of $5 \cdot 10^{-4}$ M caused an inhibition of nearly 80 per cent. The Saturn stimulates only in a strong dilution at 10^{-9} M, but this value remains below both the effect measured with the root ATP-ase and the degree of increase caused by Synprán—111.

We found that the pre-incubation with herbicides gave more favourable possibilities for the measurement of activity than the cultivation in a herbicide. Presumably the epidermis of the root lets through the herbicides only in a limited extent while during the pre-incubation the enzyme directly touches the active ingredient. In the case of plants grown in a solution containing herbicide we must reckon with some accumulation, too.

On investigations with herbicides the fact cannot be neglected that the substance to be worked up contains pure active ingredient only in a certain percentage and the other components are unknown to us. The phenomena observed can partly be attributed to the active ingredient only: besides this, some ballast materials are present and show a certain activity.

As to the ATP-ases prepared from plant and especially from animal cells, numerous references came to light about the increasing effect of detergents on the enzyme activity. The Lubrol—pX, Triton, and NaDOC are generally known as activating agents of membrane-bound enzymes. As the results prove, the activity of these substances appears in alteration of the lipid-protein connection, and this, quasi releasing the enzyme, relatively increases the protein content. It may be that also the ballast materials of the two herbicides we investigated show their activity in this

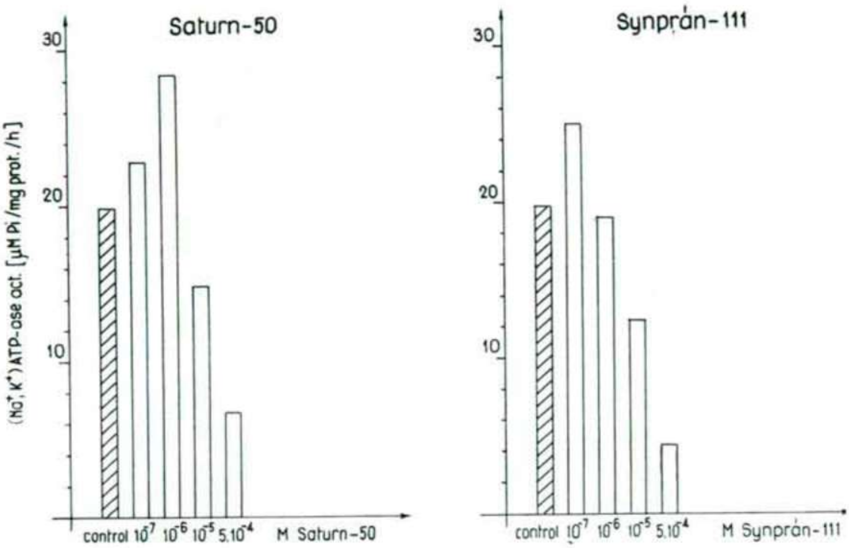

Fig. 3. Herbicide-sensitivity of ATP-ase prepared from the rice shoots.

way. In order to be able to decide this question, not only the pure active ingredients should be investigated but also the ballast substances, with a view to their effects.

As mentioned in the introduction, the active ingredients of Synprán—111 are the 2, 4, 5-T and the DCPA. The biochemical activity of the compound 2, 4, 5-T is under discussion question even today. KASAMO (1973) described that the corn coleoptyl ATP-ase was stimulated by the 2, 4, 5-T, and this is supported also by our results. As for the ATP-ase prepared from roots, especially from those of rice plants, no similar results of research are known, according to our knowledge, till now. The interpretation of the activity of Synprán is made difficult by the presence of DCPA, the enzymic effect of which is not clear yet.

As a consequence of the above, the activity of the pure effective ingredient of herbicides on the membrane structure in plant cells and further the enzyme kinetic questions (stimulation and inhibition) of the ATP-ases, must also be investigated in the future.

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